U. S. DEPARTMENT OF AGRICULTURE.
OFFICE OF EXPERIMENT STATIONS.

THE CARBOHYDRATES OF WHEAT, MAIZE, FLOUR, AND BREAD,

AND

THE ACTION OF ENZYMIC FERMENTS UPON STARCHES OF DIFFERENT ORIGIN.

BY

WINTHROP E. STONE, Ph. D.,
PROFESSOR OF CHEMISTRY, PURDUE UNIVERSITY.

WASHINGTON:
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in this work. In his work on enzymic ferments Professor Stone was assisted by students in Purdue University, as follows: Miss Isabel Henkel in the study of diastase, Mr. C. L. Meek in the study of the salivary enzyms, and Mr. W. F. Julien in the study of the pancreatic enzyms.

Professor Stone's report is submitted, with the recommendation that it be published as Bulletin No. 34 of this Office.

Respectfully,

A. C. TRUE,
Director.

HON. J. STERLING MORTON,
Secretary of Agriculture.

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THE CARBOHYDRATES OF WHEAT, MAIZE, FLOUR, AND BREAD.

THE CARBOHYDRATES OF WHEAT, MAIZE, AND FLOUR.

The class of substances known as carbohydrates constitutes a large proportion of all cereal grains. Under this head are the sugars of different kinds; the various forms of starch in its normal condition, as well as in the soluble forms, such as soluble starch, dextrins, etc.; the gums; and finally the fiber, or cellulose, which also comprises several different chemical substances. The natural grains in their air-dry state contain from 60 to 70 per cent, the fine flours and meals from 70 to 80 per cent, of these collective carbohydrates, according to the usual manner of determining the chemical composition of food stuffs.[1] By this method of analysis the data for carbohydrates are not obtained by direct determinations, but by difference, that portion of the material not otherwise determined to be of the nature of fat, ash, moisture, fiber, or of nitrogenous character being classified as "nitrogen-free extract matter" and regarded as of carbohydrate nature.

This method of estimation admits of no conclusion whatsoever as to the character of these carbohydrates, although it has frequently been pointed out that some are greatly superior to others in nutritive value.[2]

The cereal grains, with flour and meal prepared from them, have frequently been the objects of chemical analysis, but the results of these analyses have been, almost without exception, stated in the general way just referred to. Occasionally investigations have been undertaken with the purpose of separating and identifying some one constituent of the different grains, but these researches have been exceptional and isolated in comparison with the large number of general analyses. It follows, therefore, that specific information with regard to the variety and amounts of the different carbohydrates present in the cereals is meager and widely scattered. Our knowledge in this field is certainly incommensurate with the importance of the subject, since without doubt the cereal grains constitute the most important food material available to the human race.

Aside from the knowledge of the character and quantity of the carbohydrates present in grains, it also seems desirable to trace the effect

[1] U. S. Dept. Agr., Office of Experiment Stations Bul. 11.

[2] Stone, Amer. Chem. Jour., 14 (1892), p. 9; Ber. deut. chem. Ges., 25 (1892), p. 563. Stone and Jones, Agl. Sci., 7 (1893), p. 6. Ebstein, Arch. path. Anat., 129 (1892), p. 451. Salskowski, Centbl. med. Wissensch., 31 (1893), p. 193. H. Weiske, Ztschr. physiol. Chem., 20, p. 489.

of the separation of the grain into its parts, as occurs in milling, which must necessarily affect the proportions of the various constituents in the refined products; and also the changes which result in the same during the processes of cooking and baking, since the action of yeast, as in the "raising" of bread, and of heat, as in baking, are known to produce very important transformations in many of these substances.

The investigations here recorded have been prosecuted along these lines and include studies of the two cereals of most common use in North America, viz, wheat and maize, or Indian corn.

MATERIALS EMPLOYED.

For the purpose of this investigation it was thought more desirable to study carefully a few of the principal types of grains than to attempt to include a large number of samples, especially since it has appeared, wherever extended investigations of such grains have been carried on, that the variations due to varieties, climate, etc., are variations of degree and not of kind of the constituents. As typical specimens of wheat, therefore, were selected a No. 1 hard spring wheat, grown in the Northwest and employed by the Pillsbury Milling Company, of Minneapolis, for the production of their best grades of flour, and a sample of winter wheat, known as "Michigan Amber," grown on the experiment station fields at Purdue University. Representing the flour products of these two classes of wheat, there were selected "Pillsbury's Best," flour of the highest grade, made by perfected roller-process methods from the spring wheat of the Northwest, and flour designated as "Silver Moon," made from Indiana winter wheat by roller process, but of a somewhat lower grade than the Pillsbury product. This flour represents from 50 to 60 per cent of the grain and corresponds well with the quality of flour in most common use, particularly by bakers. The maize was a variety of yellow dent corn grown at the experiment station at Purdue University. It was of a more solid character than most dent varieties, and probably represents a fair average between the dent and flint varieties.

These grains were ground to uniform fineness and, with the flours, were subjected to a systematic examination as to the character of the carbohydrates which they contained. Bread was also prepared from each according to the usual methods and examined in the same way.

The materials studied comprised, therefore:

Grains and flours:

(1) Spring wheat grown in Minnesota.
(2) Winter wheat grown in Indiana.
(3) Indian corn grown in Indiana.
(4) Fine flour, "Pillsbury's Best," from spring wheat grown in Minnesota.
(5) Flour of fair grade, "Silver Moon," from winter wheat grown in Indiana.

Baked products:

(6) Bread made from spring wheat (1).
(7) Bread made from winter wheat (2).
(8) Corn cake made from Indian corn (3).
(9) Bread made from fine flour of spring wheat (4).
(10) Bread made from fine flour of winter wheat (5).

GENERAL COMPOSITION OF THE MATERIALS STUDIED.

As a basis of comparison between these and other materials of similar character, a complete food analysis was made of all the above samples. The methods employed in these analyses were in general those in common use and recommended by the Association of Official Agricultural Chemists,[1] with slight exceptions, as follows:

Moisture.—Determined by drying 2 grams of the material on a watch glass in a water oven at 98°–99°[2] during four hours.

Ash.—By incinerating in a muffle at low red heat until the ash was white.

Fat.—By extracting 2 grams of material previously dried with anhydrous ether in Soxhlet's apparatus eight to twelve hours.

Fiber.—By boiling 2 grams of material for thirty minutes successively with 200 cubic centimeters of 1.25 per cent solutions of sulphuric acid and sodium hydroxid, finally drying in a water oven, weighing, igniting, and deducting weight of the ash.

Crude protein.—By determining nitrogen by Kjeldahl's method and multiplying by the factor 6.25.

Nitrogen-free extract.—By difference, deducting the sum of percentages of all other constituents from 100.

The following table sets forth the results of these analyses, both as applying to the materials in their original or air-dry condition and also calculated to absolute dry matter:

Analyses of wheat, flour, and maize meal.

Name of material.	Air-dry material.						In dry matter.				
	Water.	Ash.	Fat.	Fiber.	Protein.	Nitrogen-free extract.	Ash.	Fat.	Fiber.	Protein.	Nitrogen-free extract.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Winter wheat	6.28	2.14	1.83	2.85	14.68	72.22	2.28	1.95	3.04	15.66	77.07
Spring wheat	8.55	1.43	2.00	2.77	14.22	71.03	1.57	2.18	3.03	15.56	77.66
Flour, winter wheat	9.92	.31	1.04	.21	12.37	76.15	.35	1.15	.23	13.73	84.54
Flour, spring wheat	9.44	.47	.91	.24	13.28	75.66	.51	1.02	.27	14.66	83.54
Meal from maize	12.43	1.51	3.80	2.35	11.60	68.31	1.72	4.34	2.67	13.25	78.02

A comparison of these data with those given for the average composition of wheat and corn[3] shows the samples under consideration to be not materially different from the average of their kind.

[1] U. S. Dept. Agr., Division of Chemistry Bul. 46.

[2] Here, as elsewhere in this article, the centigrade scale is meant.

[3] U. S. Dept. Agr., Office of Experiment Stations Bul. 11.

The wheat grain is composed of several distinct parts or portions, each possessing some particular function in the propagation of the wheat plant, as, for instance, the germ, the surrounding endosperm, and the different enveloping seed coats. Naturally these different parts of the seed exhibit differing compositions according to their offices. The outside coatings are fibrous and contain more mineral matters than other portions of the grain; the endosperm contains large amounts of starch and is the portion utilized in making fine flour; and finally the germ contains a large proportion of albuminous substances and oils. Naturally the carbohydrates may also be expected to vary in these different parts not only in character but in amounts.

The seed coats of wheat, which are rejected in the form of bran in the manufacture of fine flour, consist mainly of cellulose or fiber in which have been deposited salts and coloring matters. A large proportion of the total cellulose of the grain is to be found in these outer coats, the inclosed parts containing the minimum amounts of this carbohydrate. Wheat bran as produced in American flouring mills contains on the average 8.1 per cent of cellulose,[1] while Jago records an analysis of an English bran containing 18.3 per cent of cellulose, but this is probably an extreme case. This cellulose is regarded as belonging to the true celluloses, not affected by dilute acids, in distinction from the hemicelluloses, which are readily changed under these conditions.[2]

Starch is not normally present in the coats of the wheat grain, although frequently occurring in bran through imperfect separation from the endosperm. Especially to be noted as a characteristic carbohydrate of wheat bran is the germ-like substance yielding upon inversion with dilute acids the two pentose sugars, arabinose and xylose. This material, belonging to the class of amorphous carbohydrates known as pentosans, seems to be a complex of araban, the mother substance of arabinose, and xylan, the mother substance of xylose. It has been called araboxylan.[3]

The amount of this carbohydrate in wheat bran has been estimated at 21.9 per cent. By treating bran with dilute acids, Steiger and Schulze[4] obtained an appreciable amount of crystallized arabinose and xylose from this araboxylan constituent.

The endosperm of the wheat kernel consists of cells, the walls of which are composed of a peculiar kind of cellulose much more easily dissolved than that of the bran.[5] Inclosed in these cells is the starch,

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[1] U. S. Dept. Agr., Office of Experiment Stations Bul. 11.

[2] E. Schulze, Ber. deut. chem. Ges., 23 (1890), p. 2579; 24 (1891), p. 2227; Ztschr. physiol. Chem., 16, p. 386; 19, p. 38. Winterstein, Ibid., 17, p. 391.

[3] Steiger and Schulze, Ber. deut. chem. Ges., 23 (1890), p. 3110.

[4] Ber. deut. chem. Ges., 23 (1890), p. 3110.

[5] Jago, Science and Art of Bread Making, pp. 75, 273.

comprising the most abundant and important carbohydrate of the grain. This starch is variously stated as amounting to from 60 to 70 per cent of the weight of the grain, but these estimates have usually been made "by difference." Probably these two carbohydrates are the only ones present in the endosperm in appreciable amounts.

In the germ are probably located the sugars known to be contained in wheat and indeed a small amount of raffinose has actually been identified here.[1]

Aside from these carbohydrates known to exist in definite regions of the wheat grain, others are recorded as being present, without further reference to their location, as follows: α and β amylan,[2] two related amorphous substances, the one insoluble and the other soluble in cold water, both strongly levorotatory and capable of being converted into pentose sugars.[3] It is probable that these bodies as described are of mixed character and at least are not present in sufficient amounts to be of great importance. Galacto-xylan, a gum like amorphous substance, is said to be present in wheat and other cereals and to be converted by acids into galactose and xylose, although with some difficulty.[4]

In unripe wheat and other grains a substance called levosin has been found[5] which is soluble in alcohol and water, amorphous, levorotatory, and easily changed into sugar by the action of dilute acids or boiling water.

Not long since the assertion was made that no sugar as such was to be found in the cereal grains,[6] but in several instances careful investigation has proven the contrary. Sucrose has been isolated from wheat and identified as such.[7] The presence of invert sugar in wheat has been occasionally noted,[8] but its occurrence is to be regarded as exceptional and probably abnormal.

METHODS EMPLOYED FOR THE ESTIMATION OF CARBOHYDRATES.

In order to separate the carbohydrates of the wheat grain and to quantitatively determine the same, it was necessary, to some extent, to devise new methods, or rather new applications of methods already known, for the treatment of the material. In the following outline of the methods employed it will be noted that each constituent is actually determined and that no result is obtained "by difference." This method, moreover, brings each carbohydrate, with the exception of cellulose,

[1] Richardson and Crampton, Ber. deut. chem. Ges., 19 (1886), p. 1180. Schulze and Frankfurt, Ibid., 27 (1894), p. 64.

[2] O'Sullivan, Chem. News, 44 (1880), p. 258.

[3] Lintner, Ztschr. angew. Chem., 1890, p. 519.

[4] Lintner and Düll, Ztschr. angew. Chem., 1891, p. 538.

[5] Müntz, Compt. Rend., 87 (1878), p. 679. Tanret, Ibid., 112 (1891), p. 293

[6] A. von Asboth, Chem. Ztg., 12 (1888), pp. 25, 53.

[7] Schulze and Frankfurt, Ber. deut. chem. Ges., 27 (1894), p. 62; Ztschr. physiol. Chem., 44, p. 100; also O'Sullivan, Chem. Ztg., 9 (1885), p. 1806.

[8] O'Sullivan, Chem. Ztg., 9 (1885), p. 1806.

ultimately to the form of reducing sugar, in which state it has been determined by titration with Fehling's solution. In this way it is thought that fairly accurate information has been obtained as to the character and amounts of the various carbohydrates in the materials examined.

Sugars.—The materials were finely ground. One hundred grams was extracted with 500 cubic centimeters of boiling alcohol of 95 per cent strength during three hours, the alcoholic extract filtered, evaporated nearly to dryness, and then taken up with a small amount of water, thus freeing the sugar from the oils and waxes dissolved by the alcohol. This watery solution was invariably found to be dextrorotatory. In one case it reduced Fehling's solution slightly, but otherwise contained no reducing sugar. After inversion with a few drops of hydrochloric acid at 65° for one-half hour the solutions reduced strongly, and the sugar was estimated in the usual way with Fehling's solution, the results being calculated to sucrose.

Dextrin.—The residue from the above alcoholic extraction received 500 cubic centimeters of cold distilled water and was allowed to digest from 18 to 24 hours at ordinary temperature with frequent agitation. Upon filtering a clear solution was obtained which gave no starch reaction with iodin. This solution was slowly concentrated to a small volume, avoiding a temperature higher than 80°–90°, and was then treated with several volumes of strong alcohol until no further precipitation was produced. The flocculent precipitate thus obtained was collected, dried, and weighed; a portion was inverted with hydrochloric acid and the sugar thus obtained estimated by Fehling's solution, the results being calculated to dextrin.

Starch.—The residue from the preceding treatment was brought to a state of known dryness and its quantitative relation to the original sample established. It was now free of all matters soluble in both alcohol and cold water. The starch which was unchanged by the previous treatment was next determined. For this purpose 2 grams was accurately weighed and subjected to the action of malt extract. As is well known, the diastase contained in malt is capable of converting starch into sugars. This action occurs much more rapidly with starch paste than with raw starch, and on this account the 2 grams of material, to which had been added 100 cubic centimeters of water, was heated thoroughly for one hour by immersion in a boiling-water bath. After cooling to about 60°, 10 cubic centimeters of filtered infusion of fresh-ground malt (10 grams to 50 cubic centimeters of water) was added, and the whole maintained at a uniform temperature of 60° for one hour. After filtering and washing the residue thoroughly with hot water, the solution received 1 cubic centimeter of hydrochloric acid and was placed in a boiling-water bath one-half hour. In this way was insured a complete conversion into dextrin of all the products of the action of the malt extract upon the starch paste. The solution was then made up to

a volume of 200 cubic centimeters and the sugar determined by Fehling's solution, the results being calculated to starch by multiplying by the factor .90, after making the necessary correction for the sugar introduced in the malt extract. It will be observed that this method is such as to insure complete solution and conversion of the starch into sugar without subjecting the other carbohydrates in the sample to any more powerful reagent than boiling water and malt extract.

Pentosans and hemicelluloses.—By the successive treatments already described the more easily soluble carbohydrates will be removed and there will remain in the residue the true cellulose and the associated substances variously identified as pentosans and hexosans, or, as E. Schulze considers them, "hemicelluloses." These bodies are not soluble in water, are readily soluble in dilute alkalies, and, by the action of dilute acids, are converted into sugars of the pentose or hexose series. Arabinose, xylose, dextrose, and mannose have all been prepared from this class of carbohydrates. With regard to the particular nature of these substances occurring in wheat, not much definite knowledge is at hand beyond the fact that arabinose and xylose have been obtained from the bran. It can not be far from correct, therefore, to regard the larger part of these more susceptible bodies in wheat as pentosans. The estimation of these bodies has been effected by hydrolysis with very dilute acids and titration of the sugar thus obtained by Fehling's solution.

The residue from the preceding steps received 100 cubic centimeters of 1 per cent hydrochloric acid and was boiled one hour. This treatment has been found by numerous experiments not yet published to be sufficient to convert all of the pentosans into sugar. Councler[1] has shown that small amounts of hydrochloric acid are exceedingly efficacious in the hydrolysis of pentosans. On the other hand, this strength of acid does not attack the true cellulose, the accepted method of determining crude cellulose involving precisely such a preliminary treatment of the material with dilute acid. After filtering and washing the residue thoroughly, the solution was made up to 200 cubic centimeters and the sugars determined. The results were calculated for xylan on the assumption that the chief sugar formed is xylose. The calculation is based on the reducing power of xylose as 4.61 milligrams for each cubic centimeter of Fehling's solution,[2] and the relation of xylan according to the formula $C_5H_8O_4$ (xylan) $+ H_2O = C_5H_{10}O_5$ (xylose).

Fiber.—The residue from the preceding processes contains the more resistant forms of cellulose as the principal or only kind of carbohydrates. Other matters soluble in alcohol, hot or cold water, or rendered soluble by diastase or by dilute acids have been removed. There remains only treatment with dilute alkali to place the residue on a comparable footing with the crude fiber obtained in the usual way.

[1] Chem. Ztg., 18 (1894), p. 1617.

[2] Stone, Amer. Chem. Jour., 13 (1891), p. 73; Ber. deut. chem. Ges., 23 (1890), p. 3791.

The residue was accordingly boiled with 200 cubic centimeters of 1.25 per cent solution of sodium hydroxid during thirty minutes, filtered, dried and weighed, ignited, and the weight of the ash deducted from the first weight.

To summarize, the wheats were subjected to successive processes which permitted the separation and quantitative estimation of sucrose, invert sugar, dextrin, starch, pentosans and hemicelluloses, and crude fiber. These bodies include all of the carbohydrates known to exist in the wheat grain in appreciable amounts, and it will be noted that no carbohydrates of the usual properties could escape estimation at one or the other steps of this analytical process.

Following is the result of the examination of the two samples of wheat according to the above method:

Carbohydrates in wheat.

	Winter wheat.		Spring wheat.	
	Air dry.	In dry matter.	Air dry.	In dry matter.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Sucrose	0.48	0.51	0.66	0.72
Invert sugar	.08	.08	None.	None.
Dextrin	.25	.27	.38	.41
Starch	28.73	30.94	27.36	30.36
Pentosans	4.54	4.54	3.94	4.37
Fiber	2.68	2.68	2.26	2.51

The occurrence of a small amount of reducing or invert sugar in the winter wheat is noteworthy on account of the question with regard to its being a normal constituent of the wheat grain.

The substance classified as "dextrin" may be identical with the α and β amylan of O'Sullivan,[1] but in the absence of more confirmatory knowledge of the character of these substances it is preferred to regard this water-soluble material as dextrin, to which general class of compounds it undoubtedly bears close relationship.

THE CARBOHYDRATES OF FINE WHEAT FLOUR.

Fine flour made from wheat will not of course contain any constituents not originally present in the entire wheat grain, but since the milling process is a selective one, certain constituents of the whole grain may be in whole or in part eliminated in the milling process and lacking in the flour. The relative proportions of the remaining constituents of the flour will evidently be changed from those of the whole grain. These facts are clearly brought out in the results given in the next table of the examinations of flours made from the types of wheat above described. The method of examination was identical with that already explained.

[1] Chem. News, 44 (1880), p. 258.

Carbohydrates in wheat flour.

	Flour from winter wheat.		Flour from spring wheat.	
	Air dry.	In dry matter.	Air dry.	In dry matter.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Sucrose	0.18	0.20	0.17	0.18
Invert sugar	None.	None.	None.	None.
Dextrin	.96	1.06	.82	.90
Starch	31.87	34.04	40.88	46.19
Pentosans	None.	None.	None.	None.
Fiber	.23	.25	.22	.25

No invert sugar appears in these flours; the sucrose is proportionally much less than in the whole grain, while the crude fiber and allied pentosans have been reduced to minimal proportions. The starch is here much increased, and the dextrin-like bodies as well. It seems probable that small amounts of the starch have been converted into the soluble, dextrin-like form by the mechanical pressure and friction of the milling process. It is known that starch is susceptible to a greater or less degree to such agencies, and it is quite reasonable to ascribe the small increased amount of the dextrin in these flours to this source.

THE CARBOHYDRATES OF MAIZE.

The Indian corn or maize contains a large amount of starch and is one of the most important commercial sources of the same, particularly in America.

Washburn and Tollens[1] succeeded in isolating and identifying sucrose in the crystallized state in common maize as well as in the variety known as sweet corn. The occurrence of invert sugar was also noted. In one variety of sweet corn was found 1.47 per cent of invert sugar and 2.26 per cent of sucrose, but in the common kinds of field corn less than 1 per cent of total sugar was found.

An examination was made by Washburn's method at the Massachusetts Experiment Station[2] of 27 samples, representing 15 standard varieties of sweet corn. The corn was collected in the milk stage and carefully air-dried before analysis. The results as regards sugar content were as follows:

Sugar in sweet corn.

	Invert sugar.	Sucrose.	Total sugar.
	Per cent.	*Per cent.*	*Per cent.*
Maximum	2.93	5.60	8.53
Minimum	.69	.78	2.87
Average	1.76	3.51	5.27

The presence of pentosans in maize has been verified by several observers. As in the case of the wheat, these bodies seem to be deposited

[1] Ann. Chem., 257 (1890), p. 156; Marcacci, Centbl. agr. Chem., 19 (1890), p. 352.
[2] Massachusetts Hatch Sta. Bul. 7.

in the seed coats in close relation to the fiber or cellulose. In the bran obtained by milling maize the writer[1] showed the presence of pentosans to the extent of 5 to 6 per cent. Tollens and Flint[2] by improved methods estimated the amount to be 38.17 per cent. Schulze[3] has shown that this material was in the main xylan.

These special researches have all been confirmed in the experimental study here reported. In addition an appreciable amount of the water-soluble, dextrin-like substance already mentioned in connection with wheat was found.

The method of the examination was the same as that followed with wheat and already described. The results follow:

Carbohydrates in maize.

	Maize.	
	Air dry.	In dry matter.
	Per cent.	*Per cent.*
Sucrose	0.24	0.27
Invert sugar	None.	None.
Dextrin	.28	.32
Starch	37.28	42.50
Pentosans	4.99	5.14
Fiber	1.93	1.99

Noteworthy among these results are the absence of invert sugar and the considerable amount of pentosans present.

SUMMARY.

Wheat of both classes contains small amounts of sucrose, ranging from 0.5 per cent upward, but invert sugar is not an invariable constituent. A soluble carbohydrate not starch is present to a slight extent. The principal carbohydrate is starch, which in the samples examined reached 30 per cent. The pentosans or hemicelluloses compose over 4 per cent of the dry matter and exceed the fiber, or true cellulose, in weight. Flour produced from this wheat undergoes a total loss of pentosans and a great decrease in fiber, or cellulose, which change may be traced to the removal of the bran. The sugars have also been diminished, while the proportion of starch has been greatly increased, reaching from 35 to 45 per cent in round numbers. Starch is evidently the principal carbohydrate of fine flour.

The feed varieties of maize contain less than one-half per cent of sucrose and no invert sugar. Small amounts of the dextrin-like substance similar to that of wheat are present. Starch is abundant, equaling or exceeding the amount found in wheat. Here also the pentosans exceed the fiber, or true cellulose, nearly threefold.

[1] Stone, Amer. Chem. Jour., 13 (1891), p. 73; Ber. deut. chem. Ges., 23 (1890), p. 3791.

[2] Ber. deut. chem. Ges., 25 (1892), p. 2916.

[3] Ztschr. physiol. Chem., 19, p. 38.

THE CARBOHYDRATES OF BREAD MADE FROM WHEAT, WHEAT FLOUR, AND MAIZE.

GENERAL DISCUSSION.

When bread is prepared several agencies act upon the constituents of the flour or meal employed. In the main these agencies are destructive and the tendency is to decompose or diminish the carbohydrates. These changes may be undesirable in so far as they occasion loss of material, or changes of material into less desirable or less nutritious forms. In the main, however, the effects of baking bread are understood to be beneficial so far as their general result upon the constituents of the meal or flour is concerned. One of the chief objects of this investigation has been to determine the nature and extent of these changes, as well as to gain more knowledge as to the actual character and amounts of carbohydrates in bread. For the purposes of this study bread making with the aid of yeast has been investigated, since this method includes several processes not occurring in bread making by means of artificial leavening agents, such as baking powder.

Flour or meal made into bread by the successive processes of moistening with water or other similar agents, raising with yeast, and finally baking, is subjected to three effective agencies so far as the carbohydrates are concerned, viz, (1) the action of enzyms; (2) the action of microscopic organisms, such as yeasts, bacteria, etc.; and (3) the action of heat.

The general character and results of these agencies may be briefly discussed as follows:

The action of enzyms.—Starch-bearing seeds contain invariably, so far as studied, certain unorganized ferments, or enzyms, which are capable of converting the starch into soluble forms, thus rendering it available as soluble food for the growth of a germinating seed. These enzyms are most notable in the cereal grains, and later investigations indicate that each species possesses a specific enzym. Heretofore the enzym of barley, or *diastase*, has served as the type of this class of bodies. Whether this be identical with the enzym of wheat is still a question. Experiments made by Lintner and Eckhardt indicated that the enzym of raw wheat is different from that of malt, or diastase. The former has little action upon raw starch, but converts soluble starch into dextrin and sugar rapidly. Mention is also made of a specific enzym of maize occurring in the hard or horny part of the endosperm.[1] These enzyms become active during the process of germination, and it appears that moisture is the exciting cause of this activity, from which it follows that whenever the raw grain or the flour prepared from it is moistened, as in the preparation of the bread, conditions are produced for the beginning of diastase action upon starch.

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[1] Beyerinck, Chem. Ztg., 19 (1895), Repert., p. 144.

Just to what degree this action proceeds in the brief exposure of flour to moisture during the preparation of bread it is impossible to say, since the enzym is rendered ineffective by a temperature exceeding 50° to 75° and its action is therefore checked as soon as baking begins. It is nevertheless probably correct to enumerate this among other agencies acting upon starch to convert it into soluble forms in bread making.

The yeast employed for raising bread contains also a specific enzym, called *invertase*, capable of inverting many hundred times its own weight of sucrose and producing the fermentable derivative, glucose. This enzym acts rapidly and must speedily diminish the amount of sucrose originally present in the meal or flour when yeast is employed in the preparation of bread.

A third enzym, *cystase*, is present in grains, and during the process of germination exercises the function of dissolving the tender cellulose of the endosperm.[1] It is probable, however, that this is developed somewhat later in germination and acts more slowly than the previously named enzym; hence it is hardly to be considered as playing any rôle in the preparation of bread.

The action of yeasts and bacteria.—Yeast has been employed for the purpose of leavening or raising bread for a longer time than history can determine. Briefly stated, the action of yeast is known as alcoholic fermentation, and consists in consuming or assimilating glucose, or invert sugar, changing it into alcohol and carbonic acid, and excreting these as waste products. The theories as to the exact physiological processes involved have been many and are probably not yet wholly understood. The results are, however, clearly as stated. In bread making the yeast produces alcohol and carbonic acid gas at the expense of the sugars which are present in the flour, and the escaping gas bubbles produce the desired porosity in the dough, or "bread sponge." The yeasts are unable to produce fermentation from any other substances than certain kinds of sugars, among which sucrose is not included. Mention has already been made of the conversion of sucrose into fermentable invert sugar by the invertase of yeast, but it is doubtful if the small amount of sucrose present in the grains or flour and meal would be sufficient to produce, through the agency of the yeast, the desired degree of fermentation and leavening in bread. The enzymic conversion of starch into sugars also contributes to this process beyond a doubt. So that the action of yeast in raising bread is at the cost of the greater part of the sugars and to some extent of the starches of the flour or meal. The amount of this loss will be considered later.

The ordinary yeast employed in bread making consists in the main of the specific organism *Saccharomyces cerevisiæ*, but other varieties frequently are found mingled with this, sometimes to a considerable extent. In the main, however, the action of different yeasts upon the

[1] Brown and Morris, Jour. Chem. Soc., 62 (1890), p. 458.

carbohydrates differs only in degree. Commercial yeasts, however, frequently contain large numbers of bacteria of various kinds, particularly if the yeast is not fresh, and these organisms are also capable of attacking, dissolving, and changing the carbohydrates.

Wortman,[1] in extensive investigations, has shown that certain bacteria act upon solid or liquefied starch in a manner similar to diastase; this action is probably due to an enzym secreted by the bacteria. Wheat starch was found to be most readily affected by bacteria, as compared with other varieties. Other writers describe specific bacteria known to contribute to the fermentation in raising bread.[2] Direct comparisons as to the leavening power of *Saccharomyces cerevisiæ* and *Bacillus subtilis* have shown that the latter, when acting alone in pure cultures, is capable of setting up fermentation in flour and producing a fairly satisfactory degree of aeration in the dough.[3]

It appears, therefore, that the use of ordinary yeast for raising bread exposes the sugars of flour to rapid fermentation and the starch to solvent or saccharifying changes. The raising of bread in this way is, moreover, not a simple process, but involves the specific, characteristic fermentative and enzymic actions of oftentimes a considerable variety of organisms.

The action of heat.—In the baking process the constituents of the bread are exposed to a varying temperature according to their location in different parts of the loaf. Very little is on record as to the temperature attained in the oven during baking. Jago says that the temperature should be from 220° to 260°. Probably the ordinary temperature of the bake oven is over 200°. The exterior of the loaf is exposed fully to this degree of heat, but the interior, owing to the large amount of moisture present, can scarcely be heated beyond the boiling point of water. Some direct observations made upon the temperature of the interior of the loaf have by means of maximum recording thermometers embedded in the dough shown that it does not exceed 101° to 102°.[4] In different parts of the loaf, therefore, the carbohydrates are exposed in a moist condition to a temperature ranging from 100° to 250°. Under these conditions, particularly at the higher temperature, sucrose would undergo slight inversion, but invert sugar would not be affected. The chief change would be produced in starch. When suspended in water all varieties of starch are converted into soluble forms at a temperature of 100° or less. This action becomes less marked where the starch is merely moist. Dry starch is said to be converted into soluble forms at 200°. According to these views the starch of bread should be largely rendered soluble by the heat of baking. That

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[1] Jago, Science and Art of Bread Making, p. 188.

[2] Chicanard, Compt. Rend., 96 (1883), p. 1585; E. Laurent, Bul. Acad. Roy. Sci. Belg., ser. 3, 10 (1885), p. 765; Wigand, Entstehung u. Fermentwirkung der Bakterien, 2d ed., 1884, p. 11; Marcano, Compt. Rend., 97 (1883), p. 1070.

[3] Katherine Golden, Bot. Gaz., 15 (1890), p. 204.

[4] Balland, Compt. Rend., 117 (1893), p. 519; also A. Girard, Ibid., p. 584.

this is not the case is pointed out by Jago,[1] and also appears from the experimental data presented later. The small amount of soluble starch, dextrin, etc., found is doubtless produced in the crust of the loaf. The starch of the interior is hardly heated beyond 100°, and this is not sufficient in the absence of an excess of moisture to produce the change to the soluble forms. As to the effect of these conditions of heat and moisture upon the pentosans, hemicelluloses, and true celluloses but little is known. There is every reason to believe, however, that these constituents of flour undergo little or no change during the processes of preparing or baking bread.

METHODS EMPLOYED IN MAKING THE BREAD.

The five materials previously described, viz, winter wheat, spring wheat, flour from winter wheat, flour from spring wheat, and maize, were employed for the making of bread, or, in the case of maize, a kind of "corn cake." These products were prepared as nearly as possible in conformity with the best household practice, accompanied by careful observation and control of all conditions. A description of these methods and the data of observation are recorded here as follows:

From 12 to 18 ounces of the flour, or sufficient for an ordinary loaf of bread, was mixed with water to a thick paste, for which purpose rather more water by weight than flour was used. To this was added one-half of a small cake of Fleischman's compressed yeast (weighing about one-fourth ounce) previously rubbed up with a little water. Sugar, milk, and salt were omitted, in order to avoid the presence of materials having no bearing upon the questions to be studied. This dough was set in a warm place to "rise," the temperature being kept at 30° to 35°. The fermentation was apparently normal in each case, the operation lasting two to three hours, when the mass was spongy and in a condition satisfactory to an expert bread maker. The sponge was then thoroughly kneaded by hand, enough flour being added to make a stiff dough. This was then placed in a tin bread form and set aside for a second rising under the same conditions as before. This second rising lasted about one hour, when the bread was put at once into a previously heated oven and baked thoroughly, this last stage occupying from forty-five minutes to an hour. Just before removing the loaf the temperature of its interior was taken. The loaf was then weighed and set aside to dry and cool. At the end of eighteen hours it was again weighed (being then in a proper condition for use) and at once sliced and desiccated in preparation for analysis.

The treatment of the maize was that frequently followed in making corn cake or corn bread, a peculiarly American product, eaten warm. In its preparation baking powder or some similar artificial leavening agent is often used. About equal weights of water and maize meal (consisting of the whole grain ground fine) were mixed to a batter, one-

[1] Science and Art of Bread Making, p. 362.

fourth ounce of baking powder stirred in quickly, the whole poured into a flat form and baked in a hot oven for fifty-five minutes. The cake when baked was about 1½ inches in thickness, and its contents were doubtless more generally exposed to a higher temperature than in the case of the thicker loaves of bread.

The following data relating to the baking process, while not entirely pertinent to the study of the carbohydrates, are nevertheless given here on account of their apparent interest and the general absence of similar data in the literature of the subject. It is only necessary to remark that owing to the grossness of the process too great importance is not to be assigned to small numerical values, since slight losses and errors are not easily avoided.

Data relating to the preparation of breads.

	Whole winter wheat.	Whole spring wheat.	Flour, winter wheat.	Flour, spring wheat.	Whole maize.
Weight of flour used......grams..	317	468	475	532	298
Weight of water used......do....	276	333	305	326	291
Weight of yeast or baking powder......do....	7	7	7	7	7
Total weight of materials used......do....	600	808	787	865	596
Weight of loaf at second rising......do....	600	801	779	822	....
Weight of loaf after second rising......do....	574	766	779	815	....
Weight of loaf after baking......do....	517	680	716	773	461
Weight of loaf after 18 hours......do....	496	645	673	720	447
Shrinkage in rising......per cent..	4.33	5.19	1.02	5.78	....
Shrinkage in baking......do....	9.50	10.64	8	4.85	22.65
Shrinkage on standing 18 hours......do....	3.50	4.33	5.45	6.12	2.35
Total weight dry matter used......grams..	299	428	428	482	261
Total weight dry matter resulting......do....	289	386	425	470	251
Loss of dry matter......per cent..	3.34	9.83	0.7	2.49	0.8
Time of first rising......hours..	3	1.75	3.50	1.75	....
Time of second rising......minutes..	60	60	55	60	....
Time of baking......do....	35	45	45	45	55
Observed temperature of loaf......(degrees C.)..	99	99	99	99	98–99

The bread thus prepared was wholly normal in character, light, appetizing, and free from all sourness or other objectionable features. A study of the data presented above will be of interest. It appears that about three-fifths of the bread as prepared for the oven is water, and that the losses during rising and baking, which amounted to from 13 to 20 per cent, are largely due to the expulsion or evaporation of this water. From 4 to 6 per cent of loss occurred in the drying and airing of the bread for about eighteen hours. A comparison, however, of the actual dry weight of flour used and of the bread produced shows the actual loss of material to be very small. Under severe conditions Jago found that fermentation caused a loss of 2.5 per cent in flour. Other observers place this loss somewhat higher. With one exception in the above experiments the loss did not exceed 3.5 per cent. As practically conducted in bakeries the loss of material is said not to exceed 2 per cent.

This loss is of course due to the conversion of solid matter into gaseous or volatile products which are expelled by heat.

The temperature noted did not exceed at any time 99° in the center of the loaf.

COMPOSITION OF THE BREAD.

The different samples of bread already described were dried, finely ground, and prepared for the analytical examination. The ordinary food analysis was first made and is here given as a matter of record and as a basis of comparison with other similar materials.

Composition of bread from wheat and maize.

	In air-dry material.						In dry matter.				
	Water.	Ash.	Fat.	Fiber.	Protein.	Nitrogen-free extract.	Ash.	Fat.	Fiber.	Protein.	Nitrogen-free extract.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
Bread from whole winter wheat	3.07	2.33	1.22	2.86	15.70	74.82	2.40	1.25	2.95	16.20	77.20
Bread from whole spring wheat	7.46	1.69	1.24	2.80	15.26	71.55	1.82	1.34	3.02	16.49	77.33
Bread from fine flour, winter wheat	10.39	.59	.32	.44	11.94	76.32	.66	.35	.49	13.33	85.17
Bread from fine flour, spring wheat	8.00	.43	.47	.39	14.41	76.30	.47	.51	.42	15.66	82.94
Corn bread from whole maize	3.40	1.88	4.14	2.53	12.88	75.17	1.95	4.29	2.62	13.33	77.81

THE CARBOHYDRATES OF BREAD FROM WHOLE WHEAT.

The subjection of the carbohydrates of the wheat to the action of heat as in the process of baking has the effect of rendering a portion of the starch soluble in water without apparently changing its properties in any other essential particular. This condition renders it necessary to slightly vary the methods employed for the study of the uncooked grain as described on page 11.

The method as described below is the same as previously employed for the estimation of sugar, starch, pentosans, and cellulose, modified so as to permit of the differentiation between soluble starch and dextrins in the substances extracted from the bread by cold water. The several steps are as follows:

Sugars.—One hundred grams of material was extracted with boiling alcohol as already described.

Dextrin and soluble forms of starch.—The insoluble residue from the preceding steps received 500 cubic centimeters of cold water, was digested with frequent agitation during eighteen hours, and then filtered. The clear filtrate gave invariably the blue starch reaction with iodin, showing the presence of soluble starch, and contained besides this dextrin-like bodies. The filtrate was concentrated by slow evaporation to 200 cubic centimeters. An aliquot part (50 cubic centimeters) was taken for the determination of total carbohydrates by inversion by boiling with 1 cubic centimeter of hydrochloric acid in a water bath for one hour, neutralizing, filling to definite volume (200 cubic centimeters), and titrating with Fehling's solution, calculating the results to starch ($C_6H_{10}O_5$). A second aliquot portion (50 cubic centimeters) of the original solution was removed and the starch precipitated from it by the

method of von Asboth[1] by adding a measured volume of a standardized solution of barium hydroxid followed by 90 cubic centimeters of strong alcohol and enough water to fill to 200 cubic centimeters. After the subsidence of the flocculent precipitate, an aliquot part (50 cubic centimeters) was withdrawn and the excess of barium hydroxid determined by titration with standardized hydrochloric acid. This method is reliable for pure starch[2] but not for materials where the alkali comes in contact with the pentosans with which it combines as well as with starch. This objection would not obtain in the present case, since the pentosans are not soluble in cold water. It was found, however, that the results obtained were too high, often exceeding the total amount of carbohydrates present in the original solution as determined by inversion. It is difficult to explain this except on the assumption that the barium hydroxid formed compounds with the albuminous substances which were freely present in the solution. It was therefore impossible to determine the soluble starch directly in this way. It is probable, however, that all of the starch is thus removed from the solution, leaving the dextrin unchanged. An aliquot part (50 cubic centimeters) of the clear liquid above the starch precipitate was withdrawn, 1 to 2 cubic centimeters of hydrochloric acid added, and the whole heated in a boiling water bath during one hour; the liquid was then neutralized and titrated with Fehling's solution, the results being calculated as dextrin. By deducting the total amount of dextrin thus found from the total amount of water-soluble carbohydrates a value was obtained which is recorded as soluble starch.

Starch, pentosans, and cellulose.—The residue from the above extractions with alcohol and water was dried, weighed, and its quantitative relation to the originally 100 grams determined. Of this, 2 grams was used for the successive estimation of starch, pentosans, and cellulose in the manner already described.

The study of the bread made from whole wheat according to the above method gave the following results:

Carbohydrates of bread from whole wheat.

	Bread from whole winter wheat.		Bread from whole spring wheat.	
	Air dry.	In dry matter.	Air dry.	In dry matter.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Sucrose	0.014	0.014	0.042	0.05
Invert sugar	.100	.103	.294	.32
Dextrin	.660	.680	.209	.23
Soluble starch	1.330	1.370	2.19	2.36
Normal starch	26.100	27.930	24.180	27.08
Pentosans	3.890	4.160	3.960	4.34
Fiber	2.520	2.700	2.160	2.[illegible]

[1] Chem. Ztg., 12 (1888), p. 693; 13 (1889), p. 591.

[2] Stone, Jour. Amer. Chem. Soc., 16 (1894), p. 726.

A comparison of the above data with the corresponding results from the original wheat illustrates very clearly the changes which have been produced by fermentation and heat in the process of bread making. The sucrose has almost entirely disappeared, nor is its equivalent in invert sugar present, showing the entire disappearance of at least a part of the sugar. The invert sugar noted may have been derived from starch. The dextrin has been largely increased and a new decomposition product of starch appears, noted as "soluble starch." The starch itself has been diminished to the extent of about 3 per cent. This decrease is accounted for mainly in the soluble starch and dextrin, although it is probable that small amounts, by successive degeneration, have been ultimately converted into volatile products and been lost so far as analytical data go.

The more resistant carbohydrates, such as pentosans and fiber, as might be expected, reveal no appreciable diminution.

THE CARBOHYDRATES OF BREAD FROM WHEAT FLOUR.

The method of study applied to the bread made from fine flour was the same as that already described for the bread from whole wheat. The results of the examination are as follows:

Carbohydrates of bread from wheat flour.

	Bread from flour, winter wheat.		Bread from flour, spring wheat.	
	Air dry.	In dry matter.	Air dry.	In dry matter.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Sucrose	0.14	0.15	0.06	0.06
Invert sugar	.34	.38	.34	.37
Dextrin	.81	.91	.24	.27
Soluble starch	1.56	1.74	1.83	1.99
Normal starch	28.57	31.99	36.42	39.71
Pentosans	None.	None.	None.	None.
Fiber	.15	.17	.32	.34

The same general changes in the carbohydrates of the flour are noted here as have already been mentioned in connection with the bread from whole wheat. The sucrose has been diminished. Invert or reducing sugar not originally present in the flour is found here, possibly derived from the sucrose, or quite as likely from inverted starch. Soluble starch appears, and dextrin is also present, although it shows no constant relation to that originally present in the flour, since it is to be regarded as a transition product between starch and the fermentable sugar. Here also the starch has suffered appreciable diminution, especially marked in the case of the flour from spring wheat, where the loss amounts to upward of 7 per cent.

THE CARBOHYDRATES OF BREAD FROM WHOLE MAIZE.

According to the methods already described the following results were obtained from the study of corn bread:

Carbohydrates of bread from whole maize.

	Air dry.	In dry matter.
	Per cent	*Per cent.*
Sucrose	0.15	0.16
Invert sugar	.18	.19
Dextrin	None.	None
Soluble starch	2.69	2.80
Normal starch	38.45	40.37
Pentosans	3.37	3.54
Fiber	2.12	2.22

In the making of corn bread the carbohydrates were not exposed to the action of enzyms or any of the organized ferments (yeast or bacteria). The effect of heat is therefore alone responsible for any changes to be noted. In accordance with this we find that the sucrose has undergone partial inversion, but that the total amount of sugar has not diminished. Dextrin was not found, but soluble starch was present in considerable quantity and corresponding very closely to the loss of normal starch. The pentosans and fiber have undergone little change.

SUMMARY.

In the preparation of bread the carbohydrates of the flour or meal are subjected to various influences tending to convert them into volatile products by changing the more complex to the simpler until finally a sugar results which is fermentable. Since this process is interrupted by the heat of baking, we find in bread all successive steps of these changes. We note also the diminution of starch in appreciable quantities and the gradual disappearance of sucrose originally present in the grain and not replaced by any of the agencies mentioned. Heat also seems to play an important part in this decomposition of the starch. Nevertheless the amounts of carbohydrates thus changed are small in the aggregate. In the case of wheats, with one apparently abnormal exception, not more than 10 per cent of the total starch originally present was changed in any way. Undoubtedly this change was produced in those parts of the loaf most exposed to the full heat of the oven and not in the interior of the loaf. This leads to the conclusion that the process of baking does not change the nature or condition of the carbohydrates of wheat and maize to any important extent. This is contrary to the generally accepted belief that baking converts the starch into soluble forms to a large degree. These results are shown more clearly in the following table, where the data for the dry matter of whole grain and flour and the breads made from them are presented side by side.

Carbohydrates in dry matter of wheat, maize, flour, and bread.

	Whole winter wheat.		Whole spring wheat.		Fine flour, winter wheat.		Fine flour, spring wheat.		Maize.	
	Raw grain.	Bread.	Raw grain.	Bread.	Raw flour.	Bread.	Raw flour.	Bread.	Raw grain.	Bread.
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Sucrose	0.51	0.014	0.72	0.05	0.20	0.15	0.18	0.06	0.27	0.16
Invert sugar	.08	.10	None.	.32	None.	.38	None.	.37	None.	.19
Dextrin	.27	.68	.41	.23	1.06	.91	.90	.27	.32	None.
Soluble starch	None.	1.37	None.	2.36	None.	1.74	None.	1.99	None.	2.80
Normal starch	30.94	27.93	30.36	27.08	34.04	31.99	46.19	39.71	42.50	40.37
Pentosans	4.54	4.16	4.37	4.34	None.	None.	None.	None.	5.14	3.54
Crude fiber	2.68	2.70	2.51	2.42	.25	.17	.25	.34	1.99	2.22
Total	39.02	36.96	38.37	36.80	35.55	35.34	47.52	42.74	50.22	49.23

THE TOTAL AMOUNTS OF CARBOHYDRATES IN WHEAT AND MAIZE.

Reference has already been made to the current analytical statements of the composition of the cereal grains, which represent them as composed largely of nitrogen-free extract matters. For wheat and maize the average of all published American analyses[1] gives 70 per cent in round numbers of these constituents. For purposes of estimating feeding values or nutritive ratios this entire class of constituents is regarded as of carbohydrate nature and of homogeneous character. Frequently the analytical statement refers to these constituents directly as carbohydrates, and in the publications on the cereal grains, flour, etc., this is the ordinary way of recording those constituents not otherwise directly determined.[2] Based on these statements, the belief has become current that the cereal grains contain from 70 per cent upward of carbohydrates, although the foundation for this rests not on specific analytical determination, but on estimates "by difference." Occasionally analyses are recorded in which the sugars have been actually determined, and deducting these from the total nitrogen-free extract the difference has been called starch. It is evident to most chemists that there are grave errors in these methods, both of estimating and regarding these abundant and important constituents of foods. The results given in this paper go far to confirm this belief and to emphasize the necessity of attention to this matter.

An inspection of the last table, in which are presented the percentages of various carbohydrates calculated to the absolute dry matter of the grain, shows that the total amounts of these substances in the wheats were from 38 to 39 per cent; in the fine flours from 35 to 48 per cent, and in the maize 50 per cent. Reference to the methods employed for these determinations will show that none of the known carbohydrates could escape detection, and that the amount of error can not be great. For instance, the same material is successively extracted with boiling alcohol and cold water, treated with malt extract to dissolve starch,

[1] U. S. Dept. Agr., Office of Experiment Stations Bul. 11.

[2] Jago, Science and Art of Bread Making; Richardson, U. S. Dept. Agr., Division of Chemistry Buls. 1, 4, and 9.

hydrolysed with dilute acid, and finally the residue weighed as fiber. This method not only permits the separation of the more delicate and easily decomposed carbohydrates from those which offer greater resistance to reagents, but from the very beginning of the process any carbohydrate not wholly removed at any particular step would hardly fail of being detected at the next succeeding and more searching reaction. For instance, any sugar not removed by alcohol would be dissolved by the successive treatment with water. Any soluble compounds not secured by treatment with cold water would hardly escape the treatment with boiling water and digestion with malt extract. Again, small amounts of starch not wholly liquefied by the malt extract and removed from the cellulose tissue would certainly be converted into sugar and be detected in the subsequent boiling with dilute acid in the estimation of pentosans. Thus while small errors might occur in the separation of the carbohydrates, as has been undertaken in this investigation, it is hardly possible that any body of carbohydrate nature corresponding to sugars, starch or its soluble derivatives, pentosans, hemicelluloses, hydrolysable gums, fiber, etc., could escape detection. Without going into a discussion of the analytical methods employed, which is reserved for another place, and the use of more extensive data, it is considered pertinent to the subject under discussion to call attention to the apparent discrepancy between less than 50 per cent of carbohydrates found in our most prominent cereal grains by direct and fairly accurate methods of determination and the 70 to 80 per cent commonly ascribed to them by the indirect method of estimating "by difference." From 20 to 30 per cent of the grain or flour is not accounted for. Under the conditions this material can not be conceived of as possessing a similar nature to the sugars, starches, or even the more easily soluble forms of gums and celluloses. If it be of carbohydrate nature at all, which seems questionable, it can hardly be regarded as belonging to those substances which are measurably digestible or possessed of nutritive value.

Certainly any estimation of the nutritive character of grains, flour, or bread which ignores these facts is gravely erroneous.

RESULTS DERIVED FROM THIS INVESTIGATION.

(1) The important cereals, wheat and maize, are shown to contain a large variety of carbohydrates, among which the following have been quantitatively determined:

Carbohydrates in wheat and maize.

	Per cent.
Sucrose	0.2 to 0.70
Invert sugar	None to .08
Dextrin	.3 to .40
Starch	30.0 to 42.00
Pentosans and hemicelluloses	4.0 to 5.00
Fiber	2.0 to 2.50

In addition to these, other observers report raffinose, dextrin-like substances, etc., not separately estimated but included in the above.

(2) Flour made from wheat undergoes a selective process which leaves the carbohydrates in different proportions than in the whole grain. Sucrose and crude fiber are notably diminished; invert sugar and pentosans disappear entirely, while the starch and dextrin are proportionally increased.

(3) When these grains or the flours made from them are subjected to the action of yeast and heat, as in baking bread, an absolute loss of carbohydrates occurs, although this loss is not great. In the materials studied this loss amounted to from 1 to 5 per cent of the total dry matter of the flour or grain.

(4) The combined action of moisture, yeast, and heat, as in preparing and baking bread, diminishes the sugar and tends to convert the starch into soluble and fermentable forms. The actual amount of starch thus changed is, however, much less than is generally supposed, averaging in the materials studied not more than 10 per cent of the total starch present. This change occurs in the more exposed portions of the loaf. In the interior the starch practically undergoes no change.

(5) The temperature of the interior of loaves of bread baked in the ordinary way was never found to exceed 99°.

(6) The total shrinkage in weight during the process of "raising," baking, and "airing" bread amounts to from 12 to 20 per cent of the total weight of materials used. This loss is, however, almost entirely in moisture which has been added to the flour and which is removed by evaporation. The total loss of solid or dry matter will not exceed 3 or 4 per cent on the average.

(7) Bread contains all of the varieties of carbohydrates found in the original flour, and in addition more or less of so-called "soluble starch" produced by the action of heat upon the normal starch.

(8) An original method of analysis has been employed which permits of a fairly accurate discrimination between the different varieties of carbohydrates and the direct estimation of each.

(9) A discrepancy amounting to 20 per cent or more of the entire material is pointed out between the actual amounts of carbohydrates found in the materials studied and the amounts indicated in many of the recorded analyses in which the nitrogen-free extract determined "by difference" has been regarded as wholly of carbohydrate matter. This discrepancy introduces large and important errors into the estimation of the nutritive value of foods based on the earlier analyses.

(10) Complete food analyses are recorded of two varieties of wheat and flour made therefrom, of maize, and of bread prepared in the usual way from each of the above.

THE ACTION OF ENZYMIC FERMENTS UPON STARCHES OF DIFFERENT ORIGIN.

GENERAL DISCUSSION.

Starch is commonly regarded as a chemical compound of constant and fixed composition represented by the formula $(C_6H_{10}O_5)$ x, its molecular weight being unknown. Starches from different sources, i. e., from different species or families of plants, are assumed to be identical in all respects so far as their chemical properties are concerned, although, as is well known, great variation occurs in the superficial physical characteristics of form and size of the granules. Under the microscope certain starches reveal a more or less distinctly regular structure in layers, which arrangement becomes characteristic of certain kinds of starch (as, for instance, that of the potato). Moreover, they possess peculiar optical qualities of double refraction. These phenomena indicate a definite structure of the granule, which, while not to be regarded as crystalline, may nevertheless be reasonably spoken of as systematic. With regard to the structure of the molecule, it is not more difficult to conceive of isomeric starches than of isomeric sugars, which latter are known to exist in considerable numbers. At least in the light of the discoveries of recent years relating to the molecular characteristics of the sugars it is not unreasonable to assume that some such peculiarities may exist in the great varieties of starches. An obstacle which has operated to prevent the development of this theory has been the peculiar nature of starches, which excludes any extensive chemical study of them in their natural condition. By reason of their insolubility it is practically impossible to study their behavior toward chemical reagents without first instituting changes which undoubtedly break up and modify the original molecular arrangement. The extreme sensitiveness of the starch molecule toward the ordinary agencies employed in chemical investigations prevents therefore, to a large degree, any study of starch as such. It may be said that aside from its empirical composition we know practically nothing of starch as a chemical compound, although its derivatives and decomposition products have received quite as much and as careful study as any other carbohydrate.

So far as the writer knows, this theory of isomerism existing in starches has not found its way into the abundant literature upon the

subject, and certainly there is at present little or no experimental foundation for such a hypothesis. Nevertheless it is not more improbable than a similar assumption regarding sugars would have been a few years ago.

It is the purpose of this paper to present certain experimental data with regard to the behavior of starches of different origin. The results of the investigation have a more practical than theoretical bearing, but in seeking for a cause which should explain the results obtained only two alternatives present themselves, one of which involves important theoretical considerations as above referred to.

The numerous researches upon starch and its products rarely specify as to the character or origin of the material employed. Apparently, with few exceptions, the investigator has been satisfied with the assumption that all starches were alike in chemical composition and properties. It is noteworthy in this connection that in no other special branch of the carbohydrate bodies does so much confusion and so frequent occurrence of opposing statements exist as in connection with starch. If it be true that different isomeric forms of starch exist, it would doubtless account for this unfortunate condition of our knowledge. Occasionally, however, an investigator has called attention to the different behavior of different starches under the same conditions. These differences have been variously ascribed to the conditions and stage of development of the starch and to variations in the agencies employed. Attention has also been called to the possibility that starch is not a homogeneous substance of constant chemical composition, but an organized mixture of a variable number of chemical individuals which offer a varying resistance to outside agencies.[1] To these views is now added the thought that we may search for the cause of variation in the behavior of different starches in their molecular structure.

The attention of investigators has of late been increasingly directed to the action of enzyms upon carbohydrates. Recent researches indicate the existence of many specific enzyms, each with a peculiar ability to dissolve or decompose some particular class or series of carbohydrates. For instance, the ordinary yeast yields an enzym which inverts sucrose and maltose, but does not affect lactose; on the other hand, the enzym of the peculiar "Kefir" yeast inverts lactose.[2] Certain other yeasts produce enzyms inverting either sucrose or lactose, but not the other. Of chief interest in this place is the observation that the action of enzyms on sugar is modified or depends upon the molecular configuration of the latter.[3] It has also been noted by Fischer, and is of special interest in this place, that alcoholic fermentation is produced by yeast only in those sugars containing three, or a multiple of three, carbon atoms in the molecule.

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[1] Dafert, Landw. Vers. Stat., 33 (1886), p. 259; Bourquelot, Compt. Rend., **104** (1887), p. 177.

[2] E. Fischer and P. Lintner, Ber. deut. chem. Ges., 28 (1895), p. 984.

[3] E. Fischer; Lintner and Kröber, Ber. deut. chem. Ges., 28 (1895), p. 1429.

Such enzyms present, therefore, peculiar advantages for the study of starch, since their action is specific and without doubt less violent than many chemical reagents, the products are fairly definite, and as it now appears they act selectively, depending upon molecular configuration.

The following studies were made for the purpose of comparing the comparative susceptibility of different starches to the more important enzyms, viz, those occurring in grains, or, more especially, *diastase;* those occurring in saliva, particularly *ptyalin*, and those occurring in the pancreatic secretion. In addition, a brief study has been made of the new diastatic enzym developed by the fungus *Eurotium oryzæ*, and known commercially as "Taka-Koji" or "Taka-diastase." The results of these studies have a practical bearing as throwing light upon the comparative digestibility of different starches. They also suggest important theoretical questions as already pointed out.

MATERIALS EMPLOYED.

In selecting materials for these studies the object kept in mind was to employ those of common occurrence and abundant use as foods and at the same time representing different families or types of vegetation.

Five kinds of starch were chosen as fulfilling these requirements, viz: (1) Maize (*Zea mays*); (2) wheat (*Triticum vulgare*); (3) rice (*Oryza sativa*); (4) potato (*Solanum tuberosum*); and (5) sweet potato (*Batatas edulis*). A new portion of these starches was prepared for the experiments with each of the different enzyms as described.

These starches were prepared in the laboratory in order to prevent any possibility of mistake as to their identity. The grains or tubers were ground or grated finely and strained through muslin, by which the coarser and fibrous parts of the materials were excluded. The starch thus suspended in water was repeatedly washed by decantation until all soluble matters were removed and the water remained quite clear. At this stage the starch settled rapidly to the bottom of the vessels and was of a fine white appearance.

In the preparation of wheat starch it was necessary previously to knead the flour by hand in an excess of water to remove the gluten. The different preparations were spread out in a warm place to dry and afterwards left exposed to the air some days at ordinary temperature to enable the materials to absorb the normal amount of hygroscopic moisture. The materials were then tightly bottled to prevent any further change of weight.

The enzyms were prepared in the laboratory. As a source of diastase, freshly prepared malt was ground so as to pass through a sieve of one millimeter mesh; weighed quantities of this were digested with definite amounts of cold distilled water in the preparation of a fresh malt infusion for each series of experiments. As a source of ptyalin, human saliva was employed, representing the combined secretion of all the salivary glands. This saliva was supplied from the same person

for all the experiments, fresh portions being taken each day. The pancreatin employed was in part in the form of commercial preparations as sold by the pharmacists and in part prepared from the pancreas of freshly killed beeves and swine.

THE ACTION OF DIASTASE UPON STARCHES OF DIFFERENT ORIGIN.

GENERAL DISCUSSION.

During the germination of starch-bearing seeds, particularly the cereal grains, there is developed a substance which has the power of converting the starch into soluble forms, thus rendering it available as food for the young plant. This substance, called diastase, may be extracted from the partially germinated seed by water or glycerin, from which solution it is precipitated by alcohol as a friable white powder, which may be preserved and retains its power for a considerable time. Frequent attempts have been made to prepare this body in the pure form with a view to ascertaining its chemical composition and other properties. This has thus far been impossible, or at least the various analyses on record differ so much as to lead to the conclusion that the diastase was impure or is not of constant composition. It contains carbon, hydrogen, nitrogen, oxygen, sulphur, phosphorus, and ash materials. Diastase coagulates from watery solution on heating; possesses optical activity; when dry it withstands a temperature of 160°;[1] but in watery solution loses its power to dissolve starch when heated above 76°. Its action upon starch is increased by the presence of minimal amounts of acids as well as small amounts of salts. On the other hand, its action is hindered or completely checked by greater amounts of acids, by the smallest amounts of alkalis as well as alkaline salts. Most organic compounds are of indifferent influence, but formic aldehyde is in the highest degree a preventive of its action. The products of its action on starch when they have accumulated beyond a certain concentration also restrain the working of diastase. The ability of diastase to convert large amounts of starch into soluble compounds is remarkable. Different numbers are given by different authors, some estimations reaching so high as 200,000 times its own weight. When starch is exposed to the action of diastase it is said to undergo a series of successive decompositions, resulting ultimately in maltose. The nature and number of the various intermediate products have been the subject of much discussion. The later investigations tend toward the view that these products are fewer in number.[2] They probably consist of the various forms of dextrin, which gradually become changed to maltose and isomaltose. All of these bodies are soluble in water and hence more easily assimilated and digested than

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[1] In this article all temperatures are expressed in degrees Centigrade.

[2] Brown and Morris, Jour. Chem. Soc., 67 (1895), p. 309; Lintner and Düll, Ber. deut. chem. Ges., 26 (1893), p. 2533.

the natural starch grain. On this account various artificial preparations of malt, malt infusion, diastase, etc., have been prepared as aids to digestion.

As regards the specific action of diastase upon different kinds of starches the literature contains slight notice, and even the data at hand do not seem to have been properly appreciated in their application to physiological chemistry.

It is said that malt diastase is capable of converting the raw starch grains of wheat, barley, and rice direct, but that it only acts upon potato starch after it has been gelatinized by heat.[1] Baranetsky confirms this result as regards potato starch, and also states that diastase saccharifies the following starches with increasing ease in the order named, viz, buckwheat, wheat, beans, acorns, chestnuts, potatoes, and rice. Lintner also records some observations showing the amounts of different kinds of starches dissolved by treatment with malt at a series of different temperatures.[2] These results show considerable variation among the different kinds of starches, but apparently with no well-defined regularity.

EXPERIMENTAL STUDY.

As it was chiefly desired to gain data for a comparison of the behavior of the different starches toward diastase without regard to the nature of the products, the method of experimentation was of a nature intended to yield comparative, rather than absolute, results. Care was taken to secure (1) identically the same physical condition of all the starches, and (2) exposure under constant conditions of temperature and dilution, to a uniform solution of diastase. The preparation of the starches has already been described. For the purposes of the various experiments a weighed quantity of each starch was placed in a flask with a given volume of water. Usually such flasks were prepared in duplicate. The whole series was placed together in a large water bath and kept at the temperature of boiling water for one-half hour in order to gelatinize the starch. The flasks and contents were then cooled to 65° and placed in a water bath which was carefully maintained at this temperature until the end of the experiment.

The solution of diastase was prepared by digesting a weighed quantity of finely ground malt with a fixed volume of distilled water at ordinary temperature, three to five hours, filtering the clear liquid and adding an equal volume thereof to each of the flasks of starch, prepared as above described. The time of this addition was noted and then by frequent testing as described below the time required for the complete conversion of the starch was ascertained.

As an index of the degree of the action of the diastase, the disappearance of the iodin reaction of the starch was selected. This reaction has the advantages of being very delicate and sensitive for each

[1] Dubrunfaut; O'Sullivan; also Kjeldahl, Ztschr. Ver. Rübenz. Ind., 31 (1881), p. 727.
[2] Chem. Centbl., 1889, II, p. 845; Jour. prakt. Chem., 2 (1834), pp. 41, 91.

test and finally of not interfering with the progress of the experiment if perchance the action of the diastase were not completed. For this purpose a stock solution of iodin was made in the usual way by dissolving a few grains of iodin in a watery solution of potassium iodid. The test was made by removing about one-half a cubic centimeter of the starch solution, with a pipette, to a watch glass or a porcelain testing plate and adding a drop of iodin solution. So long as any appreciable amount of unchanged starch remained in the preparation it was indicated by the characteristic deep-blue color. When this color could no longer be obtained by repeated tests, the end of the experiment was recorded.

For the study with diastase four starches were employed, viz, sweet potato, potato, wheat, and maize.

The experiments were conducted in series, the dilution of the starch and of the malt infusion being varied in each. The particular conditions applying to each series are described in the proper place, as follows:

Series A.—One gram of starch of each kind was heated with 50 cubic centimeters of distilled water in a boiling water bath for one-half hour and cooled to 65°. Five grams of ground malt was digested at ordinary temperature with 200 cubic centimeters of distilled water during three to four hours and filtered. Ten cubic centimeters of the filtrate was added to each of the preparations of starch. Temperature throughout the experiment was 65°.

Time required for the disappearance of the starch reaction.

Number of experiment.	Sweet potato.	Potato.	Wheat.	Maize.
	Minutes.	*Minutes.*	*Minutes.*	*Minutes.*
1	8	15	60	90
2	6	13	90	90
3	14	12	90	120
4	7	12½	75	....
5	6	13½	120	....
6	....	16	....	....
7	....	15	....	....
8	....	17	....	....
9	....	12½	....	....
10	....	14	....	....
11	....	12	....	....
12	....	17	....	....

In the case of the more rapid conversion (sweet potato) it was difficult to note the exact time of disappearance of the reaction. This was also to some extent noticeable in the case of the potato starch, and the experiments were continued with this variety in order to afford the observer opportunity for practice in recognizing the end reaction.

Series B.—One gram of each of the starches was treated with 50 cubic centimeters of diluted water in a boiling water bath during one-half hour. Then 50 cubic centimeters more of water was added and the whole thoroughly mixed. Five grams of malt was digested at ordinary temperature with 200 cubic centimeters of distilled water about

three hours, filtered, and 10 cubic centimeters of the filtrate added to each of the starch preparations. The temperature was maintained at 65° throughout.

Time required for the disappearance of the starch reaction.

Number of experiment.	Sweet potato.	Potato.	Wheat.	Maize.
	Minutes.	*Minutes.*	*Minutes.*	*Minutes.*
1	4	10	33	90
2	4	10½	36	70
3	4	12	40	115
4	3½	9	30	90
5	3½	....	....	....

The effect of diluting the starch, although the same amounts of both starch and diastase were employed, appeared to reduce the time required for its conversion. The comparative relation between the different starches, however, remained unchanged.

Series C.—One-half gram of starch was heated with 50 cubic centimeters of distilled water in a boiling water bath during one-half hour. Five grams of ground malt was digested with 200 cubic centimeters of distilled water during three to four hours at ordinary temperature, filtered, and 20 cubic centimeters of the filtrate added to each of the starch preparations. The temperature was kept at 65° constantly.

Time required for the disappearance of the starch reaction.

Number of experiment.	Sweet Potato.	Potato.	Wheat.	Maize.
	Minutes.	*Minutes.*	*Minutes.*	*Minutes.*
1	2¾	3½	30	38
2	2¾	4½	35	33
3	2¾	4	25	....
4	2½	4	30	....
5	2½	4	....	....

The effect of the more concentrated malt infusion is noticeable in the lessened time of the reaction, but the relation between the different starches remained unchanged.

Summary.—The average of results in each series of experiments are expressed in the following table:

Average time required for the complete conversion of starches by diastase.

Series.	Malt used (5 gm. in 200 cc. water).	Starch used.	Water added.	Time required for complete conversion by—			
				Sweet potato starch.	Potato starch.	Wheat starch.	Maize starch.
	Cc.	*Gm.*	*Cc.*	*Min.*	*Min.*	*Min.*	*Min.*
A	10	1.0	50	8	13	90	100
B	10	.5	100	4	10	35	90
C	20	.5	50	2¾	4	30	36

These results show very conclusively that the four varieties of starch under observation vary to a marked degree in their susceptibility to the action of diastase. In the extreme cases the maize starch required more than twenty times as long for complete conversion as that of the sweet potato and never less than twelve times as long. When it is remembered that these numbers represent averages of several experiments in each case, none of which failed to follow the same order, the results are striking beyond any previous anticipation.

THE ACTION OF SALIVARY ENZYMS UPON STARCHES OF DIFFERENT ORIGIN.

GENERAL DISCUSSION.

Apparently one of the most important enzyms concerned in the solution and digestion of starch by animals is contained in the secretions of the salivary glands. This body, first observed by Berzelius, has been called ptyalin. Like other similar ferments, it is composed essentially of the elements carbon, hydrogen, oxygen, nitrogen, and ash materials. Although sulphur may be present, it is doubtful whether it is a necessary constituent. The analytical data relating to its composition are, however, variable for different samples, and the question as to the purity of such preparations arises, as in the case of diastase. Ptyalin is precipitated from solution by alcohol, and forms a white, amorphous, soluble powder. This may be preserved indefinitely and withstands heat up to 110°. Its enzymic power is destroyed when its solutions are heated to upward of 70°. The optimum temperature for its action is, according to the different observers, from 38° to 40°. It converts starch into maltose with remarkable power, but is restrained or prevented in this action by more than the least traces of acids as well as alkalis or salts of alkaline reaction. Chittenden[1] and his colleagues have investigated the properties and behavior of ptyalin in great detail under varying conditions.

The saliva serves as the medium in which this enzym naturally acts. It is slightly alkaline in reaction and consists of a mixture of the secretions of the parotid, sublingual, and submaxillary glands. The amount of ptyalin varies in the products of these different glands, being most abundant in that from the sublingual. The function of the ptyalin is to convert the starch of the food with which it is mingled into sugar. Naturally this action occurs with great rapidity, since the acids present in the gastric juice must serve effectively to check the action of the ptyalin upon food after it reaches the stomach. It is said, however, that the peptones present in the stomach protect the ptyalin from this influence to some extent. The specific action of ptyalin upon particular starches has been little studied. According to Lenberg[2] the starches of rice, wheat, maize, arrowroot, and potatoes are saccharified with

[1] Amer. Chem. Jour., 3 (1881), p. 305; 4 (1882), p. 107; 5 (1883), p. 329; 7 (1885), p. 36; Chem. News, 53 (1885), pp. 109, 137, 173.

[2] Ber. deut. chem. Ges., 10 (1877), p. 76.

increasing ease in the order named. Hammersten,[1] however, ascribes a different order, as follows: Potatoes, peas, wheat, barley, oats, rye, and maize. The reasons for this are regarded as due to the different nature of the starches,[2] or to the probable dual nature of saliva, in which sometimes one, sometimes another, enzym is present in the larger proportion.[3]

In an extensive investigation into artificial digestion Stutzer and Isbert studied the successive action of diastase, ptyalin, and pancreatin upon various feeding stuffs and found them all efficient solvents of the carbohydrates.

EXPERIMENTAL STUDY.

In studying the action of the salivary enzyms upon starches of different kinds much the same methods were pursued as in the preceding experiments with diastase. Five starches, wheat, maize, rice, potato, and sweet potato, were employed in this study, being freshly prepared in the laboratory, as already described. Weighed quantities of each were placed in flasks with fixed volumes of distilled water. In one experiment raw starches were studied; in others the starches were gelatinized by submerging the flask in a boiling-water bath one-half hour. The flasks and contents were cooled to 40° and maintained at this temperature during the experiment.

The saliva was all supplied from the same person, being collected in a beaker and, after mixing, equal portions added to each of the starch preparations.

The complete conversion of the starch was recognized by the disappearance of the blue reaction with iodin as in the experiments with diastase. The conditions were varied somewhat in different series, as is noted in the proper place.

Series A. Raw starch.—One-half gram of each of the starches with 50 cubic centimeters of water and from 3 to 10 cubic centimeters of saliva were kept at 40° during eight hours without any diminution of the intensity of the iodin reaction save in the case of the potato starch, where, after seven hours, the blue color was slightly less dense. In no case, however, was there anything approaching a rapid or complete change of the starch into sugar. In all of the subsequent experiments the starch was gelatinized by heating, as described.

Series B. Gelatinized starch.—One half gram of starch with 50 cubic centimeters of water was gelatinized by heating one-half hour in a boiling water bath.

Two cubic centimeters of saliva was added to each starch preparation. The temperature was maintained constantly at 40°. In those cases where the time was extended, tests were made at intervals of five minutes.

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[1] Ber. deut. chem. Ges., 16 (1883), p. 1938.

[2] Nycander, Chem. Centbl., 1888, I, p. 221.

[3] Bourquelot, Compt. Rend., 104 (1887), p. 71.

Time required for the disappearance of the starch reaction.

Number of experiment.	Potato.	Sweet potato.	Maize.	Rice.	Wheat.
	Minutes.	*Minutes.*	*Minutes.*	*Minutes.*	*Minutes.*
1 and 2	6	135	140	380	340
	6	135	145	385	400
3 and 4	10	170	160	405	420
	10	175	165	415	430
5 and 6	6	155	160	375	390
	6	170	160	380	400

These experiments were conducted in pairs, as indicated, on successive days. The results reveal a most striking difference as regards the susceptibility of the different starches to the action of the salivary enzyms.

Series C. Gelatinized starch.—One-half gram of starch with 50 cubic centimeters of distilled water was gelatinized by heating in a boiling water bath for one-half hour. One cubic centimeter of saliva was added to each of the starch preparations. The temperature was maintained at 40°.

Time required for the disappearance of the starch reaction.

Number of experiment.	Potato.	Sweet Potato.	Maize.	Rice and wheat.
	Minutes.	*Minutes.*	*Minutes.*	
1	13	210	195	Not complete in 8 hours.
2	13	210	220	Do.

The effect of diluting the saliva is shown in the above results. With half the former concentration the time of conversion is practically doubled, while in the case of the more resistant starches of rice and wheat no end reaction was observed after eight hours.

Series D. Gelatinized starch.—One-half gram of starch with 50 cubic centimeters of water was gelatinized by heating in a boiling water bath during one-half hour. Four cubic centimeters of saliva was added to each of the starch preparations. The temperature was maintained at 40°.

Time required for the disappearance of the starch reaction.

Number of experiment.	Potato.	Sweet potato.	Maize.	Rice.	Wheat.
	Minutes.	*Minutes.*	*Minutes.*	*Minutes.*	*Minutes.*
1 and 2	4	90	120	280	385
	4	95	120	290	330
3 and 4	4	105	150	290	320
	4	120	140	305	340

These experiments were made in pairs on successive days. The effect of the greater amount of saliva is noticeable in the lessened time of conversion. The variation in the same starches on different days is probably due to slight changes in the character of the saliva. The relative time for the different starches remains unaltered however.

Series E. Gelatinized starch.—One-half gram of each starch was gelatinized by heating in a boiling water bath one-half hour with 50 cubic centimeters of water. Six cubic centimeters of saliva was added to each starch preparation. The temperature was kept at 40°. The time required for the complete conversion of the different kinds of starch was as follows: Potato 3 minutes, sweet potato 70 minutes, maize 90 minutes, rice 165 minutes, and wheat 170 minutes.

Series F. Gelatinized starch.—One-half gram of starch with 50 cubic centimeters of water was gelatinized by heating in a boiling water bath during one-half hour. Four cubic centimeters of saliva was added to each of the starch preparations. The temperature was maintained during this experiment at 50°. The time required for the complete conversion of the different kinds of starch was as follows: Potato 3 minutes, sweet potato 70 minutes, maize 65 minutes. Rice and wheat were not completely converted in 8 hours.

The effect of the higher temperature was noticeable in the lessened time of conversion of the potato, sweet potato, and maize starches, but with the extension of the time this higher temperature apparently destroyed the activity of the enzyms.

Series G. Gelatinized starch.—One-half gram of each starch with 50 cubic centimeters of water was gelatinized by heating in a boiling water bath one-half hour. Four cubic centimeters of saliva was added to each starch preparation. The temperature was increased to 60°. The time required for the complete conversion of the different kinds of starch was as follows: Potato 2 minutes, sweet potato 25 minutes, maize 35 minutes. Rice and wheat were not completely converted in 5 hours.

These results are of interest as showing that the salivary enzyms retain their power at 60°, although it is probable that the continuation of this temperature for some time destroys this power.

Summary.—The averages of all the results in each series are presented in the following table for greater convenience of comparison:

Average time required for the complete conversion of starches by salivary enzyms.

Series.	Experimental conditions.				Time required for complete conversion by—				
	Starch used.	Water used.	Saliva. used.	Temp.	Potato starch.	Sweet potato starch.	Maize starch.	Rice starch.	Wheat starch.
	Gm.	*Cc.*	*Cc.*	*Deg. C.*	*Min.*	*Min.*	*Min.*	*Min.*	*Min.*
B	0.5	50	2	40	6	140	150	375	405
C	.5	50	1	40	13	150	195	(*a*)	(*a*)
D	.5	50	4	40	4	95	120	290	325
E	.5	50	6	40	3	70	90	185	170
F	.5	50	4	50	3	70	70	(*a*)	(*a*)
G	.5	50	4	60	2	25	35	(*a*)	(*a*)

a Not completely converted after several hours.

These results are no less striking than those obtained from the experiments with diastase, the starches exhibiting in the same way a large

variation in their behavior under precisely similar conditions. The order of the starches based upon their susceptibility is somewhat changed here. The sweet potato, which was most easily converted by diastase, now follows somewhat remotely the starch of the common potato. The maize is also more quickly converted than the wheat starch, which is the reverse of the results obtained by diastase. The extreme ratio of the time of conversion is upward of 1:80 in the case of potato and wheat.

THE ACTION OF PANCREATIC ENZYMS UPON STARCHES OF DIFFERENT ORIGIN.

GENERAL DISCUSSION.

The pancreas secretes a fluid containing at least three enzymic bodies, which play an important part in the digestive processes. One of these is of a diastatic nature, i. e., is capable of converting starch into sugar, and is thought to be identical with the ptyalin of saliva. It is not easily separated from the other bodies associated with it, but the collective secretion may be extracted from the fresh gland and exhibits a threefold action upon the carbohydrates, fats, and albuminoids. The diastatic ferment is said to be more active than even ptyalin, but evidently the conditions favorable to this action are by no means so simple as in the case of diastase and ptyalin. The optimum temperature for its action is 35°, and the presence of dilute alkalis does not modify its action. One gram of the pancreatic juice of a dog containing only 0.014 gram of organic solids is said to have converted 4.672 grams of starch into sugar in one-half an hour at 35°.

Apparently there is difficulty in securing active preparations of this diastatic enzym, and little is to be found in the literature regarding its specific action upon starch.

EXPERIMENTAL STUDY.

The manner of conducting the experiments with pancreatic ferments was much the same as that already described for the experiments with saliva and malt extract.

Weighed amounts of starch, either suspended in the raw state or gelatinized by heating in definite volumes of water, received equal, fixed amounts of the various forms of pancreatic preparations. The manner of testing for the end of the reaction was the same as that employed in previous experiments. The details of each series are described as follows:

Series A. Preliminary experiments with raw wheat starch.—Varying amounts of wheat starch were weighed and suspended in 50 cubic centimeters of distilled water. To these samples were added weighed amounts of a commercial preparation of "pancreatin" as secured from the makers. The flasks containing the mixture were placed in a water

bath kept at a constant temperature of 40°. The proportions of the material used were as follows: 100 milligrams of starch with respectively 50, 100, and 200 milligrams of pancreatin; 500 milligrams of starch with respectively 50, 100, 200, 300, and 400 milligrams of pancreatin. In no case was the starch entirely changed; indeed, no appreciable change in the character of the iodin reaction could be noted after fifteen hours. From which the conclusion was drawn that raw wheat starch is not readily converted into sugar or changed by the commercial preparations of pancreatin.

The following experiments were all conducted with different kinds of starches all gelatinized by heating with water in a boiling water bath one-half hour.

Series B. Using commercial pancreatin.—Two hundred milligrams of each kind of starch with 50 cubic centimeters of water was gelatinized. One hundred milligrams of commercial pancreatin was added to each. The temperature was kept at 40°.

Time required for the disappearance of the starch reaction.

Number of experiment.	Potato.	Sweet potato.	Maize.	Rice and wheat.
	Minutes.	*Minutes.*	*Minutes.*	
1	100	172	174	Not completed in 8 hours.
2	105	184	186	Do.

Noticeably here the action is much weaker than in the other cases, as shown by the longer time required for potato starch and the failure to completely convert either rice or wheat starch. The laboratory notes also record that the sweet potato and maize starches were not entirely changed, still giving an indefinite pink or purple color with iodin at the time indicated.

Series C. Using commercial pancreatin.—The same conditions were present as in the preceding experiment, save that the amount of pancreatin was increased to 200 milligrams, with the following results:

Time required for the disappearance of the starch reaction.

Number of experiment.	Potato.	Sweet potato.	Maize.	Rice and wheat.
	Minutes.	*Minutes.*	*Minutes.*	
1	58	317	337	Not completed in 10 hours.
2	53	323	340	Do.

Even with the increased amounts of the pancreatin the action was still weak and unsatisfactory. Of the five starches, the potato alone was definitely changed and no longer gave any reaction for starch. Sweet potato and maize still gave an uncertain reaction, although much changed, while rice and wheat starch seemed entirely unchanged.

Another preparation of pancreatin from a different manufacturer gave no better results. It is probable that these pancreatin preparations,

being intended for use as peptonizing rather than as diastatic agents, retain little of the diastatic enzym. Nevertheless the results of the above experiments are entirely in harmony with those conducted with other materials as pointing out a distinct variation between the starches of different origin.

Attempts were made to obtain a preparation of the pancreatic enzym from the fresh animal organ, and the following experiments were conducted with this material. The pancreas of freshly killed animals, in one case of swine, in another of an ox, were finely ground in a mortar with clean sharp sand until the whole was reduced to a paste. Distilled water (two or three volumes) was then added and the whole thoroughly agitated at frequent intervals. After settling, the liquid portion was decanted and used in the fresh state by adding a given volume to the starch preparation, varying the amount in different series.

Series D. Using fresh pancreatic fluid of swine.—Five hundred milligrams of gelatinized starch in 50 cubic centimeters of water was used. Temperature was kept constant at 40°.

Time required for the disappearance of the starch reaction.

Amount of pancreatic fluid.	Potato.	Sweet potato.	Maize.	Rice and wheat.
Cc.	*Minutes.*	*Minutes.*	*Minutes.*	
5	50	....	(*a*)	
5	54	....	(*a*)	
10	29	79	102	Not complete in 14 hours.
10	31	70	102	Do.
15	11	41	29	Do.
15	13	40	30	Do.
20	2½	25	14	Not converted in 9 hours.

a Not complete in 15 hours.

The freshly prepared pancreatic fluid exerted in some cases quite as rapid an effect upon the potato starch as either malt infusion or saliva, but under the same conditions was unable to change the starch of wheat and rice. Apparently the solution used was somewhat dilute, but in the later experiments both potatoes and the maize starch were quickly converted.

Series E. Using fresh pancreatic fluid of the ox.—Five hundred milligrams of gelatinized starch of each kind in 50 cubic centimeters of water was used. The temperature was constant at 40°. The pancreatic fluid was prepared in the same way as previously described. In different series respectively 20 and 40 cubic centimeters of this preparation were employed. In the former case no complete conversion of the starch was obtained even after several hours. When double this amount (40 cubic centimeters) was employed a change was noted in the starches of the potato, sweet potato, and maize, as indicated by a paler color with iodin. They were not, however, completely converted, and the wheat and rice starches so far as observed had undergone little or no change.

Summary.—Under the conditions stated the pancreatic fluids seem to have in general less ability to convert starch into sugar than the

extracts of malt or saliva. So far as the purposes of this paper are concerned, the comparative susceptibility of the different starches to pancreatic enzyms is much more marked than with the other materials studied. Diastase and ptyalin rarely failed to give definite results and to completely convert all of the starches into new compounds, but the starches of wheat and rice were in no case completely or even approximately changed by the pancreatic enzym even when the other starches were quite as quickly changed as in the most speedy results obtained from diastase or ptyalin. These results are more plainly presented in the following table:

Average time required for the complete conversion of starches by pancreatic enzym.

Series.	Experimental conditions.			Time required for complete conversion by—				
	Amount of starch used.	Amount of water added.	Amount of pancreatin used.	Potato starch.	Sweet potato starch.	Maize starch.	Rice starch.	Wheat starch.
	Mgs.	*Cc.*	*Mgs.*	*Minutes.*	*Minutes.*	*Minutes.*	*Minutes.*	*Minutes.*
B	200	50	100	100	178	180	....	....
C	200	50	200	56	320	338	....	....
D	500	50	*a* 5	52	....	....	....	....
	500	50	*a* 10	30	74	102	....	....
	500	50	*a* 15	12	40	30	....	....
	500	50	*a* 20	2½	25	14	....	....

a Cubic centimeters.

THE ACTION OF THE ENZYM OF "TAKA-DIASTASE" ON STARCHES OF DIFFERENT ORIGIN.

It is well known that many species of fungi in their processes of development produce enzyms which are capable of inverting sucrose and even in some cases of converting starch or cellulose into sugar. Notable among these products is one developed by the fungus *Eurotium oryzæ*, and discovered recently by a Japanese student, Jokichi Takamine. When this fungus is allowed to develop on wheat bran it produces abundantly a kind of ferment akin to diastase. By extracting the bran with water at a certain stage of development the ferment is dissolved, and from this solution may be precipitated by alcohol the amorphous Taka-diastase or "Taka-Koji." This substance is said to possess remarkable diastatic powers, and is capable of very important industrial and scientific applications. It is claimed that Taka-diastase will convert 100 times its weight of starch into sugar under the proper conditions. Already this substance finds extensive use in the brewing and distilling industries, and promises to supplant yeast for bread making. Medicinal preparations therefrom are also suggested as valuable aids to digestion. A small amount of Taka-diastase having been received from the makers, brief experiments were made with it upon the starches described.

Starches were prepared, as already described, by heating one-half gram of each with 50 cubic centimeters of water in a boiling-water bath

one-half hour. To each of these was added 0.25 gram of the Taka-diastase and the flasks kept carefully at 40°. After over eight hours' exposure to these conditions all of the starch gave undiminished reactions with iodin.

In a second experiment one-fourth gram of each starch was gelatinized with 50 cubic centimeters of water and kept at 40°. An equal weight of the Taka-diastase (one-fourth gram) was added to each flask. The potato starch was converted and the iodin reaction completely disappeared in seven minutes. The other starches were not converted in six hours. They were allowed to stand at ordinary temperature overnight and on the following morning the starch reaction had disappeared in all.

In a later experiment, carrying out the detailed directions of the makers as to dilution, temperature, and amounts of starch to be used, it was found that corn starch was not converted in one and one-half hours, while potato starch was completely changed in thirty minutes.

From these brief and somewhat incomplete experiments with this new enzym it appears that the different starches behave toward it quite the same as toward the older and better known ferments, some being much more rapidly converted into sugar than others.

CONCLUSIONS DERIVED FROM THIS INVESTIGATION.

(1) The starches of potato, sweet potato, maize, rice, and wheat vary greatly in their susceptibility to the action of enzymic ferments.

(2) This variation reaches such a degree that under precisely the same conditions certain of the starches require eighty times as long as others for complete solution or saccharification.

(3) This variation is exhibited toward all of the common enzymic ferments studied, viz, diastase, ptyalin, pancreatin, and "Taka-diastase," in the same relative order, with slight exception.

(4) This order, beginning with the starch which is most easily changed, is, for malt extract, sweet potato, potato, wheat, and maize; for saliva, potato, sweet potato, maize, rice, and wheat; for pancreatic fluids, potato, sweet potato, maize, with wheat and rice unchanged; for "Taka-diastase" the potato was more quickly changed than any other.

(5) Certain of the experiments indicate that the rapidity of the change in particular cases is very clearly proportional to the concentration of the solution of the ferment.

(6) It seems reasonable to assume that the same relative degree of susceptibility exhibited by these starches in the experiments described would still obtain when they are subjected to the action of the same enzyms in the processes of digestion.

(7) The facts here presented have very important bearings upon industrial operations involving the use of starches, upon questions of physiology and nutrition, and upon the study of the different starches from the purely scientific standpoint.

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